



Applicants: Michael Gallo et al. ABGX-2 CIP CPA

Application No.: 09/375,924 Filed: August 17, 1999

For: GENERATION OF MODIFIED MOLECULES

WITH INCREASED SERUM HALF-LIVES Sheet 2 of 3

STEP 1: CREATION OF UNIQUE Bsu361 RESTRICTION (CCTTAGG) SITE IN 3' TERMINUS OF GAMMA-4 CONSTANT REGION:

SEQUENCE ENCODING LAST 4 AMINO ACIDS OF HUMAN GAMMA-4 CONSTANT REGION:

TCT CTG GGT AAA [SEQ ID NO.:5]

MODIFIED SEQUENCE ENCODING THE SAME AMINO ACIDS:

TCC TTA GGG AAG [SEQ ID NO.:6]

STEP 2: PCR PRIMERS UTILIZED FOR ACCOMPLISHING SUCH MODIFICATION:

PRIMER 1:

5'74 OLIGO GGG ACC CAC GGG GTG CGA GGG C (Dra III) [SEQ ID NO.:7]

PRIMER 2:

 $3^{\circ}\gamma4$ OLIGO CTT CCC TAA GGA CAT GGA GAG GCT CTT CTG TGT GTG (Bsu361) [SEQ ID NO.:8] PRIMER 3:

 $5^{\circ}\gamma_1$ OLIGO GAT TCC TTA GGG AAG GCA GAG CCC AAA TCT AGT GAC (Bsu361) [SEQ ID NO.:9] PRIMER 4:

3'γ1 OLIGO GCC GGA ATT CGG TAC GTG CCA AGC ATC CTC GTG C (EcoR I) [SEQ ID NO.:10]

STEP 3: THREE WAY LIGATION:

- a. INTRODUCE NEW Bsu36I SITE AT GAMMA 4-HINGE JUNCTION
- b. ADD HINGE AND GAMMA 1 CH2 AND CH3 DOMAINS
- C. CLONE INTO DraIII-ECORI SITES OF EXPRESSION VECTOR (VDJ-IgG4)

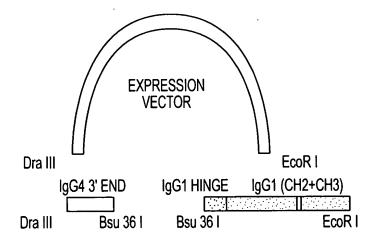


FIG. 2



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Competition for binding with protein AHRP

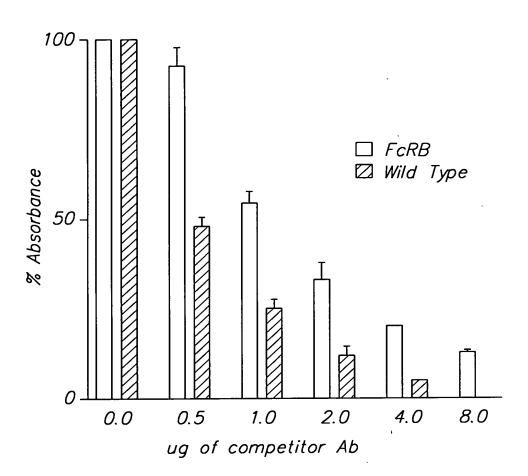


FIG. 3